THE DAILY LIFE OF A PROTOZOAN: A STUDY IN COMPARATIVE PSYCHO-PHYSIOLOGY.

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The life of an animal, as we attempt to study its physiology, appears to consist of a fabric of interwoven rhythms. Circulation, respiration, daily activity and rest, as well as reproduction and to all appearances many processes of nutrition, muscular contraction and the transmission of nerve impulse, all have come to take the form of waves or rhythms, which differ greatly in period and at certain points are interdependent. The waves of external nature, into the midst of which an animal's life is cast, no doubt tend to cause rhythmic responses on the part of the animal. This is well exemplified in the evident relation between seasonal and lunar periods and reproductive rhythms, and in the rest or activity of day and night. That external changes are not the sole determinants of physiological processes is amply demonstrated, however, by the fact that different animals living under similar environment, possess widely different rhythms. Certain controlling factors must, therefore, be sought for within the animal itself, and clearly these must be closely related to physical structure and endowment. In other words, we could not expect an organism to respond to stimuli unless it possesses mechanisms by which the stimuli may be felt. Or, conversely, if an animal responds to changes in external nature we must suppose the existence of mechanisms for their perception, although specialized structures may not have been demonstrated. To ascertain to what extent physiological processes are in fundamental character rhythmical and to be able to learn approximately what normal rhythms are, will require continuous observation of a series of animals, each for a considerable period. A series of such observations would naturally begin with the simplest animals, the protozoa.

While taking other rhythms into careful account, the present research had for its primary object a study of the rhythm of rest and activity in one of the protozoa. Since in these simple organisms, consisting of but a single cell, there are found all the important physiological, and, for all we are able to observe, types of all the psychic processes which take place in the life of one of the higher animals, it would seem possible to observe directly all the steps in the fatigue of gland, muscle or nerve which are demonstrated in more complicated bodies by indirect methods. We should be able to see, during a period of rest, zymogen granules forming and being stored up, the body grow, and possibly the nucleus increase in size. Following this, if the life of a protozoan is similar to that of higher animals in these respects, we should have a period of activity, in which new food is secured, while the body is emptied of its formed materials. If the life of a protozoan is found to be cast on rhythms similar to those of higher animals, the fact will be most remarkable, since their physical equipment is so different. If protozoan rhythms prove to be strikingly dissimilar, in what ways may such peculiarities be correlated with differences in structure?

The work here described was done in the physiological laboratory of Clark University, in the fall of 1893. For much assistance in construction of apparatus, we wish to express our thanks to Mr. J. R. Slonaker.

Among the numerous protozoa available, the Vorticella[1] was chosen for two reasons. First, being attached permanently by its stalk, a specimen can be retained in the field of a microscope for days without difficulty. This is, of course, a prime condition of the experiment. Second, the animal is active, its movements are well defined and easily observed and some of them appear to be clearly automatic, others, purposeful and selective. These movements may be classified as follows:

AUTOMATIC.

1. Contraction of vesicle.
2. Ingestion of food balls.
3. Ejection of detritus.

PSYCHO-REFLEX.

1. Contraction of stalk, with attendant closure of bell.
2. Vibration of peristomal cilia.
3. Sorting of particles by the the sensory cilia, the driving of food toward the mouth, and the driving away of waste particles.

[1]Descriptions of the Vorticella are so readily accessible in all manuals of biology and zoology that no attempt to describe it here is deemed necessary.

Objection may be raised to placing ciliary activities upon the psychic side, and it is true that the action of cilia in various parts of the human body could not be considered of such character. On the other hand, the work of the cilia in Vorticellæ seems to be at every point more complicated than that of ordinary cilia. By their movements currents are set up with the apparent purpose of drawing food within reach. When a particle is touched by the cilia, an act of choice is apparent, and in accordance to this choice the particle is carried toward the mouth or whirled away. This process would seem to indicate no less conscious wakeful action on the part of Vorticellæ than the seeking of prey and the feeding of animals in general. No account is taken of the extension of the stalk, since on the view that this is due entirely to elasticity of the cuticle it is a purely mechanical action.

Apparatus was devised to record the occurrence of all these phenomena. It consisted of a continuous-roll kymograph with eight capillary glass pens arranged vertically across the paper. The lowest pen was connected with an electro-magnet and the hands of a clock so as to register hours and minutes. Two other pens registered temperature and barometric pressure, and the five remaining were attached to tambours in such a way that the observer could record various activities by a touch of the fingers of the right hand, without taking his eye from the microscope. The first recorded contractions of the stalk; the second, contractions of the vesicle; the third, ingestion of food particles; the fourth, ejection of detritus; the fifth, reproductive phases. The observer's left hand remained free to adjust the focus of the microscope.

The microscope used was a Zeiss, apochromatic series, ocular 6, objective 4 mm., which gave a magnification of 375 diameters. In order to keep the Vorticellæ under conditions as normal as possible, a stream of water from an aquarium, in which various plants were growing, was kept flowing under the cover-slip. This was accomplished by means of a glass syphon, drawn to a capillary point, placed at one side of the cover-slip, and a filter-paper drip applied to the other side.

The first Vorticella observed corresponded to the species *gracilis*, as indicated in W. Saville Kent's "Manual of the Infusoria." It was observed, without a moment's intermission, between 8 and 8.30 and between 9.30 A. M., Nov. 3 and 6.30 A. M., Nov. 4, and every contraction of the stalk or vesicle registered upon the kymograph paper. The following table shows its life history for the whole period of observation. The times given in the first column are usually

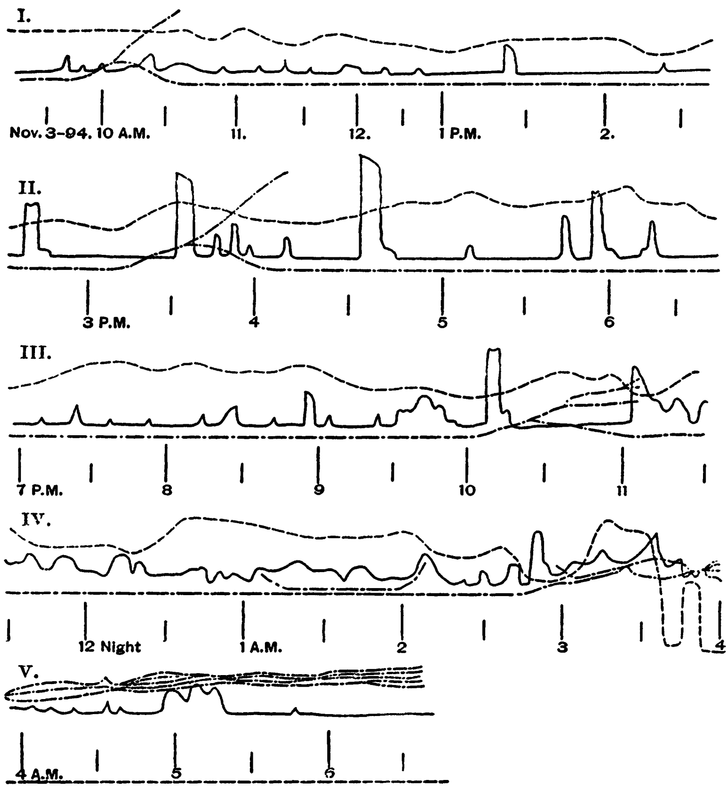
TABLE.

Experiment 1. Vorticella gracilis.

Date.	Time.	Contraction of Vesicle per min.	Contraction of Stalk.	Remarks.
Nov. 3,	8.00 A. M.	3		
"	8.30 "	2.8	8 in 30 mins.	No rhythm.
"	9.30 "	2.6	1 in 8 (about).	
"	10.00 "	2.6	1 in 8 (about).	Division begins.
"	10.13 "	2.6	1 per min.	Two vesicles visible.
"	10.33 "	2.6	Several in rapid succession.	Division complete.
"	10.35 "	2.6	Less frequent and less violent after division.	
"	11.35 "	2.6		
"	11.55 "	2.6		
"	12.00 M.,	3.2	Rare.	
"	12.15 P. M.	2.4		
"	12.52–1.50	2.4	None.	
"	2.00 P. M.	2		
"	2.50 "	2.4	Several in rapid succession.	No stimulus apparent.
"	3.15 "	2.2	None in 25 mins.	
"	3.33 "	2.8	2.8 per min. (about) for 5 mins.	Second division begins.
"	3.42 "	2.8	1.5 per min. (about).	Two vesicles visible.
"	4.11 "	2.8	1.5 per min.	Division complete.
"	4.50 "	2.8	1.5 per min.	No observable stimulus.
"	5.00 " to	Slowly varying between 2.2	Varying irregularly, rare.	Gas light. No visible change in contractions of stalk or vesicle.
"	10.00 "			
"	10.21 "		None.	Third division begins.
"	10.35 "		None.	Subdivision of daughter cell begins.
"	11.00 "		None.	Further subdivision of daughter cell begins.
"	11.05 "	and 3.2	Rapid contractions.	Daughter cells detached.
Nov. 4,	1.15 A. M.	2.7	Feeble contractions.	Free-swimming zoid in contact.
"	2.10 "	2.4		
"	2.12 "	1.6		Vesicle much swollen.
"	3.06 "	1d8		Free-swimming zoid becomes attached.
"	3.15 "	3		
"	3.25 "			Vesicle suddenly divides.
"	3.46 "		The two vesicles and that of the free-swimming zoid all break into one which contracts five or six times, at the rate of 1.5 per minute. After this no more contractions of vesicle can be seen. Following this a curious succession of partial divisions and reunions occurred, lasting until 6.30 A. M. See Figure.	
"	3.52 "	1.5		
"	5.44 "		Last contraction of stalk.	

those at which greatest changes appear in the rhythms recorded.

The course of this Vorticella's life may be seen graphically represented in cut below. The lines in this figure are plotted directly from the kymograph record, but to avoid complication the coördinates, excepting designations of time in hours and half hours, have been omitted. The continuous line represents stalk contractions. For somewhat more than half its extent it is at its lowest level, indicating that no contractions occurred during that time. Elevations are drawn proportionate in height to the number of contractions occurring during the time traversed. The broken line indicates in a similar way frequency of vesicle-contractions, the higher the



Experiment 1, Vorticella gracilis. The continuous and broken lines represent frequency of stalk and vesicle-contraction respectively. The line of dots and dashes indicates reproductive phases. For further explanation, see Table above.

curve, the more frequent the beats. It reaches its zero point when the vesicle ceases to contract. The line of dots and dashes represents reproductive phases, its rise and division indicating increase in size and division of the Vorticella. The time at which one of the bells detaches itself from the stalk upon completion of divisions is marked by the termination of the corresponding line. Conjugation is indicated by the approach and union of a similar line.

No account has been taken of movements of the cilia, for the simple reason that they are not a variable in the animal's life. During the whole period of observation the cilia were working incessently, drawing particles toward the mouth, sorting them, ingesting food and driving away excreta and debris. An apparent exception to this statement occurs at the instant of a stalk contraction, when the bell is also contracted into a sphere and the cilia are drawn in; but during this operation there is only time for the cilia to fold in and open out again, and hence there is nothing which could be construed as a period of rest. The same statement applies to the results of ciliary activity, viz., ingestion of food and ejection of detritus. In other words, so far as our observation goes, there is practically continuous action of cilia and, in consequence, a practically constant stream of particles both into the body and out from it.

As stated above, the temperature and barometric pressure were traced upon the kymograph paper along with the Vorticella's activities. This was done in order to ascertain whether these physical factors had any influence similar to their influence upon the tissues of higher animals. These tracings are omitted from the chart and from further consideration, because no hint of any connection or causal correspondence could be made out. As far as temperature is concerned, sudden change from ice water to water at room temperature (20-22 C.), or vice versa, never was observed to act as a stimulus sufficient to occasion stalk-contractions, nor did the rhythm of the contractile vesicle appear to be influenced in the slightest degree. This would seem to indicate that a vorticella is not endowed with even the rudiments of temperature sense. It is more difficult to disprove the influence of barometric pressure.

In all, fourteen experiments were made, similar to that just described, lasting from a few hours to five and a quarter days. Observation in all these experiments, especially the longer ones, was not continuous, though it was frequent during both day and night. The presence of several species of Vorticella and of Carchesium was utilized to add variety to the experiments. Farther than showing the physiological fact that the

rhythm of the contractile vesicle is somewhat different in the different species and tends to vary in a similar way under the same conditions, these experiments are simply confirmations of the first.

In Experiment 3, V. gracilis, observation was continued during the entire process of conjugation. Not the least change in the movements of the cilia, the taking in of food, etc., could be noted. Stalk contractions were frequent. During the hour in which the process was completed (9.42-10.48 A. M.) the vesicle-contractions gradually decreased in frequency from 8 to 2.6 per minute. Shortly after conjugation (11.09 A. M.) the stalk remained closely contracted and the bell detached itself and its movements could be no longer followed.

In later experiments by attending more carefully to food supply and by preventing as far as possible the growth of mould and bacteria and keeping the stream of water under the cover-slip as clean as possible, we were able to keep the Vorticellæ in apparently much more normal condition for a longer time. In spite of all efforts and precautions, however, mould and bacteria sooner or later overran the field and either killed the Vorticellæ or compelled them to migrate. We attempted to obviate this difficulty by sterilizing the water supply, and by boiling and covering antiseptically, at the same time giving in the place of their normal food a pure culture of yeast plants. This attempt resulted in an interesting demonstration of the educability of Vorticellæ. At first they took this, to them, newly discovered food with great avidity, filling their bodies to distention with food vacuoles of the yeast. In a very few minutes, however, the entire meal was ejected with volcanic energy. Not a single torula was allowed to remain in the body, and for several hours at least—how long the memory lasted was not determined—the individual could not be induced to repeat the experiment.

Experiment 11 was continued for two days and was terminated by accident. Experiment 14, upon V. campanula, lasted five and a quarter days and leaves no ground for doubting the truth of our main conclusion, viz., that a Vorticella works continuously and shows in its life no period of inactivity or rest corresponding to periods of rest in higher animals. In other words, a Vorticella never sleeps.

During the five days, frequent observation both day and night failed to detect any considerable relaxation of apparent effort or attention. The cilia worked uniformly, drawing in food particles and sorting them. In fact all efforts to surfeit the tiny animals with food produced no appreciable effect in satisfying their apparent hunger. Division occurred

frequently, but only in rare cases did this cause any noticeable relaxation of other work. Occasionally for a few moments during the act of division the bells became nearly spherical and their cilia worked feebly and in one instance ceased vibrating altogether. This had the appearance of a momentary "rest" but its occurrence was a rare exception to the rule of continuous work throughout the process of division. No instance of conjugation occurred in this experiment and this suggests a point of importance that has not as yet received attention. Under certain conditions a Vorticella passes into what is known as the encysted state, in which the bell becomes spherical, detaches itself from the stalk and secretes a cyst. It now is said to "rest" or "lie dormant" through a period of such unfavorable conditions as dryness or cold, and when circumstances favoring activity again return, it bursts its cyst, not as a single zoid, but as a number of small free swimming zoids. The stage of rest or encystment is thus also a period of reproduction. Each of the minute Vorticellæ attaches itself, develops a stalk and grows to the normal size of the species. Just the bearing which this phase of a Vorticella's life has upon the problem of rythmical periods of rest and activity our experiments do not determine. So far as they yield any evidence, they support the view indicated above, which is generally adopted, that conditions unfavorable to life cause this mode of reproduction, conjugation and encystment. Encystment is a means of protecting the animal from changes in its environment which would otherwise prove fatal. Upon this supposition, as long as conditions of life remain favorable, a Vorticella might continue to live and work and reproduce by division indefinitely without the intervention of a "resting" stage. Encystment is therefore of the nature of an enforced "rest," a period of inactivity imposed by exceptional external circumstances; and therefore has no bearing upon the problem in hand.

During the course of this experiment, careful tests were made of the Vorticella's sensitiveness to vibrations of sound and light. No one can watch a Vorticella for an hour without being struck by its exceedingly delicate sense of touch. The slightest jar is instantly answered by a quick contraction of the stalk, and particles scarcely visible under the microscope are sorted with the greatest apparent precision. No less striking throughout all the experiments was a Vorticella's insensibility to all other stimuli. No reaction could be elicited to changes in light or to sounds of any kind so long as these were unaccompanied by perceptible jarring of the microscope. Musical sounds of all qualities and volumes were tried, but without effect. Bright sunlight was

flashed upon the creatures from total darkness; this was varied by interposing colored glasses, red, green, blue and violet, between the mirror and the stage of the microscope; each light was allowed to act for minutes at a time and was also tried in a succession of quick flashes, but not the least evidence of sensation could be detected. Thus, so far as we can judge, the universe must consist for a Vorticella of a series of touches, possibly also of tastes and smells; but not to any extent of sights and sounds.

Correlating now what we have learned concerning the activities of this animal with the anatomical structures at its command, we remark first that a Vorticella consists for the most part of a mechanism for digesting food. Supplementing this is a motor mechanism beautifully adapted for securing it. Material thus obtained and assimilated causes the body to grow to a certain size, but when this limit is reached the body divides instead of enlarging indefinitely. A prime condition of the creature's life must be ability to distinguish food from what is not food. This it is able to do sufficiently by the sense of touch, and the ciliary mechanism which mediates this sense is precisely similar to tactile and sensory "hairs" as they exist throughout other parts of the animal kingdom. But like any other animal a Vorticella must be able to escape from its natural enemies.

The experiments afford evidence ample to prove that this is the chief purpose of the stalk and its contractions. Several earlier experiments were suddenly terminated by a "monster" appearing in the field and snapping off the Vorticella's bell. In a number of cases contraction of its stalk actually pulled the bell out of a devourer's mouth. The particular enemy observed was a minute white worm hardly more than visible to the unaided eye. A necessary preliminary to later experiments consisted in carefully teasing all these animals out of the preparations. The sense by which a Vorticella is made aware of the approach of its enemy is touch. At least a Vorticella was never observed to react until its cilia were actually touched.

With food in abundance, capable of yielding a continuous supply of energy, it is a strange physiological paradox that all animals should not be able to work continuously. For any of the higher animals, at least, this is not possible. A certain amount of activity produces fatigue, and fatigue makes necessary a period of rest. Fatigue is commonly explained upon two assumptions. The first of these is that decomposition products arising from activity of the tissue are not removed quickly enough to avoid poisoning or clogging the organism. The second assumption is that highly organized ma-

terials are consumed during action more rapidly than they are formed. So generally are these processes present in the tissues of animals usually studied that we are apt to consider fatigue as a universal characteristic of living matter. It seems only reasonable, however, that protoplasm may be formed as fast as used under favorable conditions of nutrition, and that with equally good facilities for the removal of decomposition products, these may not accumulate in amounts sufficient to interfere with activity. So far as we are able to interpret the significance of our own experiments, this is the state of things in a Vorticella under favorable conditions of life.